International Food Research Journal 23(6): 2326-2334 (December 2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mini Review

Bioactive lipids in milk

*Dhankhar, J., Sharma, R. and Indumathi, K. P.

Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, (India)-132001

Article history

Received: 29 August 2015
Received in revised form: 10 March 2016
Accepted: 16 March 2016

Keywords

Fatty acids
Butyric acid
Trans fatty acids
Conjugated linoleic acid
Phospholipid

Abstract

Milk fat represents a significant source of nutrition and energy for humans stimulating extensive investigations dominating recent health concerns even at micro levels by bio-scientists. Despite its longstanding association with health, the current researches are directed at number of beneficial components like bioactive lipids and fat soluble vitamins. Bioactive lipids in milk include triglycerides, diglycerides, monoglycerides associated with beneficial fatty acids like short chain, medium chain, conjugated linoleic acid (CLA), and polyunsaturated fatty acids. These can prove as potential diagnostic tools for reckoning chronic diseases like cancers, diabetes, inflammation, and CVD (Cardiovascular disease). The minor lipid components like phospholipids, sterols, ether lipids also carry biological and health promoting activities. Butyric acid plays important role in controlling cell growth, differentiation and preventing tumor genesis in colon cells. Fat-soluble vitamins (A and E) in milk fat are involved in antioxidant and tumor suppressing activities. CLA has been shown to play important role in inhibition of carcinogenesis and atherosclerosis as well as immune stimulation in different studies on animals. Furthermore, phospholipids like sphingolipids and their active metabolites, ceramides and sphingosines had exhibited effective antibacterial properties, anti carcinogenic and immune stimulation properties. The current information on bioactive lipid components in milk lipids in promoting health and preventing disease has been summarized in the paper.

Introduction

Milk and dairy products are important sources of fatty acids (FA) in the human diet (Haug *et al*., 2007; Mills *et al*., 2011), contributing to between 18% and 24% total fat, 30% and 40% total saturated fatty acid (SFA) and 20% and 25% total trans fatty acid intake (Henderson *et al*., 2003). In the preceding decades, nutritionists and scientists have cautioned against high milk consumption as the high concentrations of saturated fatty acids (SFA) in milk fat had been reported to negatively impact to human health, especially with increased risk of cardiovascular disease. Nevertheless, latest reviews and epidemiological studies revealed that milk has a characteristic composition with short, medium chain, polyunsaturated, branched fatty acid and conjugated linoleic acid (CLA) that play important role in regulating biological activities. This increase in the knowledge that milk lipid components confer biological activities besides health beneficial properties, further boosted research in dairy industry for formulating the dairy products with incorporation of bioactive lipid components. The upsurge in the interest in these bioactive components has led to the different strategies for their incorporation in foods, by either isolating individual components as such or by enriching bioactive lipid component as a whole in the food products that have resultantly filliped the innovations and ramifications in the range of nutraceuticals available commercially. A deeper knowledge on the regulation of the metabolic pathways of these bioactive lipids and their potential positive effect in human health will be essential in the formulation of these products with added value.

In milk, lipids are mainly present in globules as an oil-in-water emulsion (MacGibbon and Taylor, 2006).The milk fat consists mainly of triglycerides, approximately 98%, while other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) about 0.1% (Jensen and Newburg, 1995). In addition, there are trace amounts of ether lipids, hydrocarbons, fat soluble vitamins, flavour compounds and compounds introduced by the feed (Parodi, 2008).

Triglycerides (TAG)

The triglyceride is the major component of milk fat, esterified with different fatty acids. Milk fat

*Corresponding author.
Email: *jyotika008@yahoo.com*

contains TAG with different carbon numbers varying from C26 to C54 (Fontecha *et al.*, 2005) based upon total number of carbon atoms distributed across the whole of structure of TAG molecule (i.e. the sum of the carbon atoms of the three acyl radicals).The range of variation of carbon number is species specific with greater amount of medium-chain TAG (C26-C36) in goat and sheep's milk than cow's milk and both species also exhibit lower proportion of long chain TAGs (C46-C54) (Goudjil *et al.*, 2003).The TAGs which are composed of saturated and short chain fatty acids with 6-10 carbons generally exhibit lower melting point in synergy with less energy dense as compared to TAGs with long chain fatty acids. These variations in physical and chemical properties affect the metabolism and absorption of TAGs. The esterification of fatty acids at three positions of TAG molecule is non random (MacGibbon and Taylor, 2006) with short chain acids butyric (4:0) and caproic (6:0) are esterified to the sn-3 position and the C16:0 occupies the sn-2 position.

The hydrolysis of triacylglycerols in milk, on consumption occurs with the help of lingual lipases in the mouth and by both lingual and gastric lipase in the stomach (Parodi, 2004). The lipases selectively hydrolyse sn-3 position fatty acids, and thus release the shorter fatty acids. The short chain fatty acids C4:0-10:0 are absorbed through the stomach wall in decreasing quantities with the increase in molecular weight, enter the portal vein, and are transported to the liver where they are oxidised. The 25-40% of TAG digestion takes place in stomach (Jensen, 2002).

Milk fatty acid composition

Saturated fatty acids

In physiological terms, classification of saturated fatty acids can be done as: short-chain (up to C-4:0), medium-chain (up to C-6:0-10:0), and long-chain (C-12:0 onwards) fatty acids, although different classifications are used in other contexts. On an average, C4:0-10:0 account for 10.6-12.8 weight % of total fatty acids in milk fat (Precht, 1990; Jensen and Newburg, 1995). The major five fatty acids constituting almost 70-77% of the total fatty acid composition in milk are long chain fatty acids (C18:1, C16:0, C10:0, C14:0, and C18:0). The milk fat of different species exhibits characteristic fatty acid compositions. The content of short chain fatty acids: butyric (C4:0), caproic (C6:0), caprylic (C8:0) and capric (C10:0) acids are 2-3 fold higher in sheep than in goat's milk.

The digestibility of short and medium chain fatty acids is quite high. Further, these play crucial role in reduction of cholesterol levels in serum and liver, as well as triacylglycerols levels (Frede *et al.*, 1990). They are transferred directly from the intestine to the portal circulation on hydrolysis from parent TAG without again undergoing resynthesis of triacylglycerols. Thus, they are utilised for instant energy production and are not available for adipose formation. Epidemiological studies have shown that butyric acid (4:0) is an antineoplastic agent (Parodi, 1996; Parodi, 1997). Butyric acid is reported to (i) prevent the proliferation (ii) to induce differentiation (in vitro) of a variety of neoplastic cells (Prasad, 1980; Chen *et al.*, 1994) and (iii) to retard the tumorigenesis in mice (Pouillart *et al.*, 1991). Most studies have shown synergism between butyrate and other dietary components and common drugs in decreasing cancer cell growth (Parodi, 2008). Butyric acid modulates the expression of oncogenes and suppressor genes (Rabizadeh *et al.*, 1993). The range of butyric acid in milk fat is approx. 7.5-13.0 mole % or 3.42 weight % related to fat (Molkentin and Precht, 1997). In animal and *in vitro* studies, the short and medium chain fatty acids (C4:0, C6:0, C8:0 and C10:0) had been reported to exert antibacterial and antiviral activities (Neyts *et al.*, 2000). The long-chain fatty acids 12:0-16:0 in milk fat constitute 42-44 wt % of total fatty acids (Precht, 1990; Jensen and Newburg, 1995). These long chain fatty acids are associated with increment in level of total and LDL cholesterol in plasma, which is responsible for increased risk of CVDs and atherosclerosis (Mattson and Grundy, 1985; Dupont *et al.*, 1991; ZoÈllner and Tato, 1992; Zock, 1995). However, stearic acid (C18:0) with an average concentration in milk fat of 11% has a neutral effect on lipoprotein cholesterol levels (Hegsted *et al.*, 1965; Bonanome and Grundy, 1988; Grundy and Denke, 1990). Animal studies demonstrated that butyric acid in milk fat induce uncoupling protein -1 expression in brown adipose tissue, which was associated with suppression of diet-induced obesity and improvement in insulin sensitivity (Parodi, 2016).

Monounsaturated fatty acids

The major monounsaturated fatty acid present in milk fat is oleic acid and is responsible for 20.1-20.8 wt % of total fatty acids on average (Precht, 1990; Jensen and Newburg, 1995). Most scientific studies pointed out that diets with higher oleic acid mostly decrease the level of LDL-cholesterol as compared to diets containing less oleic acid, without affecting HDL-cholesterol levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Berry *et al.*, 1991; Chan *et al.*, 1991; Mata *et al.*, 1992; Valsta *et al.*, 1992;

Katan *et al*., 1994; Zock, 1995). Thus, oleic acid can be safely regarded as antiatherogenic.

Polyunsaturated fatty acids

Milk fat also contains polyunsaturated fatty acids (PUFAs) like linoleic acid (18:2n-6) and a-linolenic acid (18:3n-3) almost to the level of 1.2-2.0 wt % and 0.5-0.7 wt % of total fatty acids, respectively (Precht, 1990; Jensen and Newburg, 1995; Precht and Molkentin, 1997). They perform many diverse functions being part of essential fatty acids in human metabolism and are conducive for physiological aberrations on deficiency (Kinsella *et al*., 1990; Horrobin, 1997).

Furthermore, evidence from many studies have shown that linoleic acid decreases the level of LDL-cholesterol but concurrently may also cause a slight decrease of HDL-cholesterol levels (Mattson and Grundy, 1985; Beynen and Katan, 1989; Mensink and Katan, 1989; Grundy and Denke, 1990; ZoÈllner and Tato, 1992; Katan *et al*., 1994). Most of the studies have concluded antiatherogenic effect of PUFA.

Although, more recent reviews have reported that PUFAs present in the triacylglycerol and cholesterol ester moieties of LDLs may be responsible for atherogenesis. PUFAs contain unsaturated bonds which get oxidized to form cytotoxic compounds in the LDLs (ox-LDLs). The LDLs on oxidation result in formation of atherosclerotic plaques in the blood vessels (Witztum and Steinberg, 1991; Holvoet and Collen, 1994; Parthasarathy and Santanam, 1994). The concentration of PUFA is remarkably high in cells of brain and retina, where it is responsible for proper mental functioning and visual acuity.

Moreover, epidemiological studies have suggested that consumption of n-6 fatty may promote tumor formation, whereas some n-3 PUFAs have been correlated with antitumorigenic effects (Cohen *et al*., 1986; Pariza, 1988; Reddy, 1994). Furthermore, PUFAs exert immunomodulatory effect by resulting in formation of anti-inflammatory cytokines ((Pfeuffer, 1997).

Conjugated linoleic acids

Conjugated linoleic acid (CLA) consists of a mixture of positional and geometric isomer of linoleic acid with conjugated double bond system (Chin et al., 1992; Precht and Molkentin, 1997). Rumenic acid (RA) or cis-9, trans-11 is the predominant isomer, out of the 20 main isomers found in dairy products (Kramer *et al*., 1994). CLA is synthesized as intermediate of polyunsaturated fatty acids (PUFAs), specifically linoleic (18:2, n-6) and linolenic acids (18:3n-3) by microflora in rumen (Palmquist *et al*., 2005; Luna *et al*., 2005). The milk fat content of CLA varies among different species, with sheep's milk having the highest content of CLA as well as that of its precursor vaccenic acid (t-11;C18:1). The content of CLA in milk fat varies species wise in the decreasing order with sheep followed by cow and goat 1.2, 0.7 and 0.6 % of total fatty acids, respectively. Several prospective studies suggested that CLA intake is related to down regulation of fat synthesis and lipogenic enzymes for lipid synthesis. CLA supplements inhibited milk fat synthesis and improved energy mass balance in studies carried out with lactating goats (Baldin *et al*., 2014). Vyas *et al.* (2013) reported that infusion of CLA resulted in depression in fatty acid synthesis in lactating cows. Abdominal infusion of CLA reduced de novo synthesized fatty acid (DNFA) to level of 50% concentration, whereas DNFA tended to be greater with Butter fat infusion (Parodi, 2016). CLA-induced milk fat depression in lactating ewes involved the sterol regulatory element-binding protein transcription factor family and a coordinated down regulation in transcript abundance for lipogenic enzymes involved in mammary lipid synthesis(Hussein *et al*., 2013).

The current rise in the interest of CLAs started with the studies suggesting association of ant carcinogenic and anti-neoplastic activity with consumption of CLA (Pariza *et al*., 2000). Several other additional physiological and pathological responses have also been ascribed to CLA including effects on several different kinds of cancers, metastases, atherosclerosis, diabetes, immunity and body fat/protein composition.

The CLA plays important role in cancer prevention by regulating cell metabolism, anti proliferative and apoptotic activities (Ochoa, 2004). Moreover, studies have shown that high CLA intake on consumption of dairy products results in reduction of risk factors associated with colon-rectal cancer (Larsson, 2005).

Furthermore, other plausible mechanisms also have been postulated to elucidate the anti-inflammatory and anticancer activity of CLA. The fatty acids CLA and arachidonic acid (C20:4) compete with each other in influencing the action of cyclooxygenase enzyme and thus resultantly reducing the concentration of pro-inflammatory compounds like prostaglandins and thromboxane of the 2-series. The reduction in activity of cyclooxygenase by CLA decreases the release of pro-inflammatory cytokines such as TNF-alpha and interleukin in animals (Akahoshi *et al*., 2004). Other mechanisms suggest activation of PPARs transcription factors that reduce the activation of NF-kappa B and thereby reducing cytokines, adhesion molecules and other inflammatory compounds induced by stress (Cheng *et al*., 2004).

Furthermore, various other CLA isomers also have emerged concomitant with regulation of several biological activities related to health. Hence, the C18:2 trans 10, cis 12, has also attracted attention lately as it has been postulated to promote weight loss (Parodi, 2004; Belury, 2002) and also with accompanying decrease in glucose levels and plasma insulin resistance (Riserus *et al*., 2002; Khanal and Dhiman, 2004).

Moreover, among other CLA isomers, the C18:2 cis 9, cis 11 has been reported to be inhibitor of estrogen signalling breast cancer in human cells in vitro assays (Tanmahasamut *et al*., 2004). While other studies have also highlighted the positive effect of C18:2 trans 9 trans 11 in controlling the growth of human colon cancer cells (Beppu *et al.*, 2006). The studies conducted on bovine endothelial cells exhibited anti proliferative and pro-apoptotic effects (Lai *et al*., 2005).

Trans fatty acids (TFA)

The TFA is synthesized in milk by rumen bacteria through hydrogenation of PUFAs and its content in milk fat varies from 2.5 to 5% of total fatty acids. The TFA content depends upon seasonal and dietary variations for ruminant species. Therefore, a diet rich in linoleic and linolenic acid coming from pasture feeding in summer leads to higher TFA contents than the diet given in winter which consists of concentrate (Henninger and Ulberth, 1994; Wolff, 1994; Precht and Molkentin, 1997).

The most common TFA present in milk fat is trans-octadecenoic acid (trans-18:1).The content of TFA shows variation in different species. Amongst ruminant, the highest amount of TFA is reported in milk fat of sheep followed by cow and goat respectively. However, the three species show similar pattern of C18:1 trans isomer distribution (Precht *et al*., 2001). Analogously, the presence of TFA in dairy fat is not correlated with good health.

However, currently TFA has come under scrutiny lens due to its adverse effect on lipid profile and on other risk factors for CVD; Resultantly the research being directed to the question as to whether industrially derived TFA has similar adverse effects as for dairy derived TFA and also whether both of them undergo similar pattern of metabolic fate or different one (Glew *et al.*, 2010). The isomers of TFA in fats of ruminant origin and partially hydrogenated vegetable oils show remarkable similarity with many isomers in common. However the isomer profile of hydrogenated vegetable fats is very different. The main isomers generated during the hydrogenation of vegetable fats consist of trans monounsaturated fatty acids (e.g. C18:1 trans 9, elaidic acid) whereas the main TFA present in milk fat is C18:1 trans 11, VA (IDF-International Dairy Federation, 2005). Though VA is a TFA, it is quite important one, as it is the precursor of the main isomer of CLA, rumenic acid (RA) which is most relevant bioactive compound present in milk fat. The modulation of VA into RA occurs both in the bovine mammary gland (Griinari and Bauman, 1999; Field, 2009) as well as in human and other animal tissues (Turpeinen *et al.*, 2002; Mosley *et al*., 2006; Kuhnt *et al*., 2006).

Scientific studies evidenced that different individual TFA isomers have different physiological effects (Anadón *et al.*, 2010). It has been reported that TFA from hydrogenated oils has adverse effect on LDL and other risk factors of atherosclerosis whereas, the predominant TFA in milk, VA, does not exhibit the deleterious effects (IDF-International Dairy Federation, 2005; Parodi, 2006). Additionally, the evidence generated from studies of Pfeuffer and Schrezenmeir (2006) showed that there was a positive correlation between the risk of CVD and the intake of TFA from hydrogenated vegetable oils. Hence, the scientific data evidenced that intake of TFA is associated with CVD risk, whereas there exists a negative or neutral correlation between animal or dairy TFA.

Minor lipid compounds

Phospholipids

The milk fat globule membrane (MFGM) contains phospholipids which constitute about 0.5-% of total milk lipids (Christie, 1987; Rombaut and Dewettinck, 2006; Rodríguez-Alcalá and Fontecha, 2010; Benoit *et al*., 2010; Donato *et al*., 2011; Le *et al*., 2011). They concluded that phospholipids like sphingolipids and their active metabolites, ceramides and sphingosines, possessed potent antimicrobial activity against pathogens. Besides, there is evidence from controlled studies that phospholipids exhibit tumor-suppressing properties by inducing cell proliferation and also display cholesterol lowering properties (Parodi, 2004; Parodi, 2006; Gustavsson *et al*., 2010). The phospholipids have been ascribed to carry anti oxidative properties in dairy fat products with low water content (Molkentin *et al.*, 2000). The distribution of phospholipids content in the different ruminant milks are similar with different fractions showing varying proportions: Phosphatidylcholine (35%) phosphatidyl-ethanolamine (30%), and sphingomyelin (25%) are the major constituents, with smaller amounts of phosphatidylinositol (5%) and phosphatidylserine (3%), (MacGibbon and Taylor, 2006; Sanchez-Juanes *et al*., 2009; Kielbowicz, 2013). The supplementation of the high-fat diet with a phospholipids-rich dairy milk extract caused a significant decrease of the liver weight, total liver lipid, liver triglycerides and total cholesterol and serum lipids in studies carried out with mice (Wat *et al*., 2009). Watanabe *et al.* (2011) also confirmed similar results. Studies conducted by Ohlsson *et al.* (2009, 2010) on humans, consisting of the supplementation of a sphingolipid-enriched dairy formulation, partially supported the findings that these molecules may affect cholesterol concentrations in TG-rich lipoproteins, but they did not find any effect on the level of plasma lipids or lipoproteins. Keller (2012) concluded that milk PL supplementations influenced the plasma cholesterol amount, but did not change the LDL/HDL ratio.

Ether lipids

The ether lipids present in milk fat are alkyldiacylglycerols and alkylacylphospholipids. Milk neutral lipids are reported to contain 0.01 wt % of 1-O-alkyldiacylglycerols and milk phospholipids to contain 0.16 wt% 1-O-alkylacylphospholipids (Hallgren *et al*., 1974). Ether lipids and their derivatives have antineoplastic tendencies (Parodi, 1996; Parodi, 1997). They possess anticarcinogenic activities against cancer cells which comprise of inhibition of growth, antimetastatic activity and induction of differentiation and apoptosis (Berdel, 1991; Diomede *et al*., 1993). It has been established that ether lipids are incorporated and accumulated in cell membranes and thereby influence biochemical and biophysical processes.

Sterols

Sterols represent about 0.25-0.45% of total fat and classified as unsaponifiable portion of milk fat. Its content depends among other things on the method of fat extraction and is believed to be near 0.4 wt % in most cases (Goudjil *et al.,* 2003). Sterols are polycyclic alcohols having a secondary –OH group at position 3, and the presence of this group makes sterols more polar than triglycerides. Minor sterols represent 3-5% of sterol content (Precht, 2001). The minor sterols are mainly intermediates formed during biosynthetic pathway of cholesterol synthesis like lanosterol, desmosterol, lathosterols, dihydrolanosterol and 7-dehydrocholesterol (Fauquant *et al*., 2007) and also include phytosterols like β-sitosterol, campesterols, brassicasterols which are present in very minor amounts and are most likely to be originating from feed (Walstra and Jennese, 1984). Cholesterol is important for the re-absorption of fats and is a precursor in the synthesis of steroid hormones. Cholesterol is negatively correlated with elevated plasma cholesterol levels along with fatty acids with regard to atherosclerosis and coronary heart diseases.

Conclusion

Milk lipids have a complex composition consisting of a variety of bioactive substances with health promoting attributes. However, milk also contains saturated fatty acids as well as cholesterol which have been negatively associated with health, making consumers skeptical about its consumption. In addition to this, health concerns and nutritional recommendations have contributed to the decline in its consumption. For this reason, global research has been promoted for revitalization of milk fat utilizing its bioactive components. Current strategies to increase CLA in BML also care for their positive correlation with TFA. However, an independent increase of CLA content could further improve the physiological properties of milk fat.

The public perception of whole milk fat dairy products is a significant challenge that faces the dairy industry because of their perceived negative effects on human health. However, milk is a complex food with host of nutrients, and the conclusions from long-term studies and meta-analyses suggest a reduction in risk in the subjects with the highest dairy consumption relative to those with the lowest intake for almost all-cause deaths and diseases. The diversity of milk fat lipids, the variety of bioactive substances that it contains and their physiological functions remains poorly understood. Therefore, further research is required to establish the contribution of these bioactive components of milk fat in human health.

References

Akahoshi, A., Koba, K., Ichinose, F., Kaneko, M., Shimoda, A., Nonaka, K., Yamasaki, M., Iwata, T., Yamauchi, Y. and Tsutsumi, K. and Sugano, M. 2004. Dietary protein modulates the effect of CLA on lipid metabolism in rats. Lipids 39: 25-30.

Anadón, A., Martínez-Larrañaga, M. R., Martínez, M. A., Ares, I., Ramos, E., Gómez-Cortés, P., Juárez, M. and Fuente, M. A. 2010. Acute oral safety study of dairy fat rich in trans-10 C18:1 versus vaccenic plus conjugated linoleic acid in rats. Food Chemistry Toxicology 48: 591-598.

Baldin M. R., Dresch, J., Souza D., Fernandes, M. A. S., Gama, K. J., Harvatine, D. E. and Oliveira, 2014. CLA induced milk fat depression reduced dry matter intake and improved energy balance in dairy goats. Small Ruminant Research 116(1): 44-50.

Belury, M. A. 2002. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annual Reviews Nutrition 22: 505-531.

Benoit, B., Fauquant, C., Daira, P., Peretti, N., Guichardant, M. and Michalski, M. C. 2010. Phospholipid species and minor sterols in French human milk. Food Chemistry 120: 684-691.

Beppu, F, Hosokawa, M., Tanaka, L., Kohno, H., Tanaka, T. and Miyashita, K. 2006. Potent inhibitory effect of trans9, trans11 isomer of conjugated linoleic acid on the growth of human colon cancer cells. Journal of Nutritional Biochemistry 17(12): 830-836.

Berdel, W. E. 1991. Membrane-interactive lipids as experimental anticancer drugs. British Journal of Cancer 64: 208-211.

Berry, E. M., Eisenberg, S., Haratz, D., Friedlander, Y., Norman, Y., Kaufmann, N. A. and Stein, Y. 1991. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins- The Jerusalem Nutrition Study: high MUFAs vs high PUFAs. American Journal of Clinical Nutrition 53: 899-907.

Beynen, A. C. and Katan, M. B. 1989. Impact of dietary cholesterol and fatty acids on serum lipids and lipoproteins in man. In Vergroesen, A. J. and Crawford, M. (Eds). Role of Fats in Human Nutrition, pp. 237-286. London: Academic Press.

Bonanome, A. and Grundy, S. M. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New England Journal of Medicine 318: 1244-1248.

Chan, J. K., Bruce, V. M. and McDonald, B. E. 1991. Dietary n-3, linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. American Journal of Clinical Nutrition 53: 1230-1234.

Chen, Z. X. and Breitman, T. R. 1994. Tributyrin: a prodrug of butyric acid for potential clinical application in differentiation therapy. Cancer Research 54: 3494-3499.

Cheng, W. L., Lii, C. K., Chen, H. W., Lin, T. H. and Liu, K. L. 2004. Contribution of conjugated linoleic acid to the suppression of inflammatory responses through the regulation of the NF-kappaB pathway. Journal of Agricultural and Food Chemistry 52: 71-8.

Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anti carcinogens. Journal of Food Composition and Analysis 5: 185-197.

Christie, W. W., Noble, R. C. and Davies, G. 1987. Phospholipids in milk and dairy products. Journal of the Society of Dairy Technology 40: 10-12.

Cohen, L. A., Thompson, D. O., Maeura, Y., Choi, K., Blank, M. E. and Rose, D. P. 1986. Dietary fat and mammary cancer. Promoting effects of different dietary fats on N-nitrosomethylurea-induced rat mammary tumorigenesis. Journal of the National Cancer Institute 77: 33-42.

Diomede, L., Colotta, F., Piovani, B., Re, F., Modest, E. J. and Salmona, M. 1993. Induction of apoptosis in human leukemic cells by the ether lipid 1-octadecyl-2-methyl-rac-glycero-3-phosphocholine: A possible basis for its selective action. International Journal of Cancer 53: 124-130.

Donato, P., Cacciola, F., Cichello, F., Russo, M., Dugo, P. and Mondello, L. 2011. Determination of phospholipids in milk samples by means of hydrophilic interaction liquid chromatography coupled to evaporative light scattering and mass spectrometry detection. Journal of Chromatography 1218: 6476-6482.

Dupont, J., White, P. J. and Feldman, E. B. 1991. Saturated and hydrogenated fats in food in relation to health. Journal of the American College of Nutrition 10: 577-592.

Fauquant, C., Briard-Bion, V., Leconte, N., Guichardant, M. and Michalski, M. C. 2007. Membrane phospholipids and sterols in micro filtered milk fat globules. European Journal Lipid Science and Technology 109: 1167-1173.

Field, C. J., Blewett, H. H., Proctor, S. and Vine, D. 2009. Human health benefits of vaccenic acid. Applied Physiology, Journal of Nutrition and Metabolism 34: 979-991.

Fontecha, J., Goudjil, H., Ríos, J. J., Fraga, M. J. and Juárez, M. 2005. Identity of the major triacylglycerols in ovine milk fat. International Dairy Journal 15: 1217-1224.

Frede, E., Precht, D. and Timmen, H. 1990. Lipide: FettsaÈuren, Fette und Fettbegleitstoffe einschlieblich fettloÈslicher Vitamine. In Kompendium zur milchwirtschaftlichen Chemie, Eschlimme. (Eds). pp. 57-78. MuÈnchen: Volkswirtschaftlicher Verlag.

Glew, R. H., Chuang, L. T., Berry, T., Okolie, H., Crossey, M. J. and VanderJagt, D. J. 2010. Lipid Profiles and trans Fatty Acids in Serum Phospholipids of Semi-nomadic Fulani in Northern Nigeria. Journal of Health Population and Nutrition 28: 159-166.

Goudjil, H., Fontecha, J., Fraga, M. J. and Juárez, M. 2003. TAG composition of ewe's milk fat: Detection of foreign fats. Journal of American Oil Chemists' Society 80: 219-222.

Goudjil, H., TORRADO, S., Fontecha, J., CASTRO, I. M., Fraga, M. J. and Juárez, M. 2003. Composition of cholesterol and its precursors in ovine milk. Le Lait 83:153-160.

Griinari, J. M. and Bauman, D. E. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W. and Nelson, G.J. (Eds). Advances in CLA Research, Volume 1, pp. 180-348. AOCS Press: Champaign, IL.

Grundy, S. M. and Denke, M. A. 1990. Dietary influences on serum lipids and lipoproteins. Journal of Lipid Research 31: 1149-1172.

Gustavsson, M., Hodgkinson, S. C., Fong, B., Norris, C., Guan, J. A., Krageloh, C. U., Breier, B. H., Davison, M., McJarrow, P. and Vickers, M. H.2010. Maternal supplementation with a complex milk lipid mixture during pregnancy and lactation alters neonatal brain lipid composition but lacks effect on cognitive function in rats. Nutrition Research 30: 279-289.

Hallgren, B., Niklasson, A., Stallberg, G. and Thorin, H. 1974. On the occurrence of 1-O-alkylglycerols and 1-O(2-methoxyalkyl)glycerols in human colostrum, human milk, cow's milk, sheep's milk, human red bone marrow, red cells, blood plasma and a uterine carcinoma. Acta Chemica Scandinavica 28: 1029-1034.

Haug, A., Hostmark, A. and Harstad, O. 2007. Bovine milk in human nutrition. Lipids in Health and Disease 6: 25. doi:10.1186/1476-511X-6-25.

Hegsted, D. M., McGandy, R. B. and Myers, M. L. 1965. Quantitative effects of dietary fat on serum cholesterol in man. American Journal of Clinical Nutrition 17: 281-295.

Henderson, L., Gregory, J. and Swan, G. 2003. The national diet and nutrition survey: Adults aged 19-64 years Vol. 2: Energy, protein, carbohydrate, fat and alcohol intake. London, UK: The Stationery Office.

Henninger, M. and Ulberth, F. 1994. Trans fatty acid content of bovine milk fat. Milchwissenschaft 49: 555-558.

Holvoet, P. and Collen, D. 1994. Oxidized lipoproteins in atherosclerosis and thrombosis. FASEB Journal 8: 1279-1284.

Horrobin, D. F. 1997. Essentiality of oils and fats for human nutrition and disease. In Shukla V. K. S. and Kochhar S. P. (Eds). Modern Developments in Food Lipids. pp. 171-192. Aarhus/Danmark: International Food Science Center (ISBN 87-894166-3-4).

Hussein, M., Harvatine, K.H., Weerasinghe, W. M. P. B., Sinclair, L. A. and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. Journal of Dairy Science Volume 96(6): 3825-3834

IDF-International Dairy Federation. 2005.Trans fatty acid: Scientific Progress and labelling. Bulletin of International Dairy Federation, 393, Brusseles, Belgium.

Jensen, R. G. 2002. The Composition of Bovine Milk Lipids: January1995 to December 2000. Journal of Dairy Science 85: 295-350.

Jensen, R.G. and Newburg, D. C. 1995. Bovine milk lipids. In Jensen, R. G. (Eds). Handbook of Milk Composition, pp. 543-575. San Diego/CF: Academic Press.

Katan, M. B., Zock, P. L. and Mensink, R. P. 1994. Effects of fat and fatty acids on blood lipids in humans: an overview. American Journal of Clinical Nutrition 60 Suppl: 1017-1022.

Keller, S., Malarski, A., Reuther, C., Kertscher, R., Kiehntopf, M. and Jahreis, G. 2012. Milk phospholipid and plant sterol-dependent modulation of plasma lipids in healthy volunteers. European Journal of Nutrition 52(3): 1169-1179.

Khanal, R. C. and Dhiman T. R. 2004. Biosynthesis of conjugated linoleic acid (CLA): A review. Pakistan Journal of Nutrition 3: 72-81.

Kielbowicz, G., Micek, P. and Wawrzenczyk, C. 2013. A new liquid chromatography method with charge aerosol detector (CAD) for the determination of phospholipids classes. Application to milk phospholipids. Talanta 105: 28-33.

Kinsella, J. E., Broughton, K. S. and Whelan, J. W. 1990. Dietary unsaturated fatty acids: Interaction and possible needs in relation to eicosanoid synthesis. Journal of Nutritional Biochemistry 1: 123-141.

Kramer, J. H., Misik, V. and Weglicki, W. B. 1994. Lipid peroxidation-derived free radical production and postischemic myocardial reperfusion injury. Annals of the New York Academy of Sciences 723: 180-196.

Kuhnt, K., Kraft, J., Moeckel, P. and Jahreis, G. 2006. Trans-11-18:1 is effectively delta9-desaturated compared with trans-1218:1 in humans. British Journal of Nutrition 95: 752-761.

Lai, K. L., Torres-Duarte, A. and Vanderhoek, J. 2005. 9-trans, 11-trans-CLA: Anti proliferative and proapoptotic effects on bovine endothelial cells. Lipids 40(11): 1107-1116.

Larsson, S. C., Bergkvist, L. and Wolk, A. 2005. High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort. The American Journal of Clinical Nutrition 82: 894-900.

Le, T. T., Miocinovic, J., Nguyen, T. M., Rombaut, R., van Camp, J. and Dewettinck, K. 2011. Improved solvent extraction procedure and high-performance liquid chromatography evaporative light-scattering detector method for analysis of polar lipids from dairy materials. Journal of Agricultural and Food Chemistry 59: 10407-10413.

Luna, P., Fontecha, J., Juárez, M. and de la Fuente, M. A. 2005. Effects of a Diet Supplemented with Linseed on the CLA Content in Ewes Milk Fat. Lipids 40: 445-454.

MacGibbon, A. H. K. and Taylor, M. W. 2006. Composition and structure of bovine milk lipids. In Fox, P. F. and McSweeney P. L. H. (Eds). Advanced dairy chemistry p.1-42. New York: Springer.

Mata, P., Garrido, J. A., Ordovas, J. M., Blazquez, E., Alvarez-Sala, L. A., Rubio, M. J., Alonso, R. and de Oya, M. 1992. Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. American Journal of Clinical Nutrition 56: 77-83.

Mattson, F. H. and Grundy S. M. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. Journal of Lipid Research 26: 194-202.

Mensink, R. P. and Katan, M. B. 1989. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. New England Journal of Medicine 321: 436-441.

Mills, S., Ross, R., Hill, C., Fitzgerald, G. and Stanton, C. 2011. Milk intelligence: Mining milk for bioactive substances associated with human health. International Dairy Journal 21: 377-401.

Molkentin, J. 2000. Occurrence and biochemical characteristics of natural bioactive substances in bovine milk lipids. British Journal of Nutrition 84: 47-53.

Molkentin, J. and Precht, D. 1997a. Representative determination of the butyric acid content in European milk fats. Milchwissenschaft 52: 82-85.

Mosley, E. E., McGuire, M. K., Williams, J. E. and McGuire, M. A. 2006. Cis-9, Trans-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women. Journal of Nutrition 136: 2297-2301.

Neyts, K., Huys, G., Uyttendaele, M., Swings, J. and Debevere, J. 2000. Incidence and identification of mesophilic Aeromonas spp. from retail foods. Lett. Journal of Applied Microbiology 31: 359-363.

Ochoa, J. J., Farquharson, A. J., Grant, I., Moffat, L. E., Heys, S. D. and Wahle, K. W. 2004. Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for cis-9, trans-305 11 and trans-10, cis-12 isomers. Carcinogenesis 25:1185-1191.

Ohlsson, L., Burling, H., Duan, R. D. and Nilsson, A. 2010. Effects of a sphingolipid-enriched dairy formulation on postprandial lipid concentrations. European Journal of Clinical Nutrition 64: 1344-1349.

Ohlsson, L., Burling, H. and Nilsson, A. 2009. Long term effects on human plasma lipoproteins of a formulation enriched in butter milk polar lipid. Lipids in Health and Diseases 8: 44. doi:10.1186/1476-511X-8-44.

Palmquist, D. L., Lock, A. L. and Shingfield, K. J. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Advances in Food and Nutrition Research 50: 179-217.

Pariza, M. W. 1988. Dietary fat and cancer risk: evidence and research needs. Annual Review of Nutrition 8: 167-183.

Pariza, M. W., Park, Y. and Cook, M. E. 2000. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proceedings of Society for Experimental Biology in Medicine 223: 8-13.

Parodi, P. 1997. Cow's milk fat components as potential anti carcinogenic agents. Journal of Nutrition 127: 1055-1060.

Parodi, P. 2004. Milk fat in human nutrition. Australian Journal of Dairy Technology 59: 3-59.

Parodi, P. W. 1996. Milk fat components: possible chemo preventive agents for cancer and other diseases. Australian Journal of Dairy Technology 51: 24-32.

Parodi, P. W. 2006. Nutritional significance of milk lipids. In Fox, P.L.H. Peter W. and Parodi McSweeney. (Eds). Advanced Dairy Chemistry, Volumen 2: Lipids, 3ª edición. pp. 601-639. Springer: EEUU.

Parodi, P. W. 2008. Milk lipids: their role as potential anti-cancer agents. Science Des Aliments 28: 44-52.

Parodi, P. W. 2016. Cooperative action of bioactive components in milk fat with PPARs may explain its anti-diabetogenic properties.Human Health and Nutrition Research, Dairy Australia. ResearchMedical Hypotheses 89: 1-7.

Parthasarathy, S. and Santanam, N. 1994. Mechanisms of oxidation antioxidants and atherosclerosis. Current Opinion in Lipidology 5: 371-375.

Pfeuffer, M. 1997. Bedeutung mehrfach ungesaÈttigter FettsaÈuren in der ErnaÈhrung. Fette in der ErnaÈhrung. In Schriftenreihe des B. M. L., Warwel, S., Weber, N. and Reihe, A. (Eds). Angewandte Wissenschaft, pp. 35-67. Heft Bonn: KoÈllen-Verlag.

Pfeuffer, M. and Schrezenmeir, J. 2006. Impact of trans fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. International Dairy Journal 16: 1383-1388.

Pouillart, P., Cerutti, I., Ronco, G., Villa, P. and Chany, C. 1991. Butyric monosaccharide ester-induced cell differentiation and anti- tumor activity in mice, importance of their prolonged biological effect for clinical applications in cancer therapy. International Journal of Cancer 49: 89-95.

Prasad, K. N. 1980. Butyric acid: a small fatty acid with diverse biological functions. Life Science 27: 1351-1358.

Precht, D. 1990. Quantitativer Nachweis von Milchfett in Schokoladenmischungen In Bestimmung von Milchfettanteilen in Kakaobutter. European Journal of Lipid Science and Technology 92: 153-161.

Precht, D. 2001. Cholesterol content in European bovine milk fats. Nahrung 45(1): 2-8.

Precht, D. and Molkentin, J. 1997. Effect of feeding on conjugated cisD9, transD11-octadecadienoic acid and other isomers of linoleic acid in bovine milk fats. Nahrung 41: 330-335.

Precht, D., Molkentin, J., Destaillats, F. and Wolf, R. L. 2001. Comparative studies on individual isomeris 18:1 acids in cow, goat and ewe milk fat by low-temperature high-resolution capillary gas-liquid chromatography. Lipids 36: 827-832.

Rabizadeh, E., Shaklai, M., Nudelman, A., Eisenbach, L. and Rephaeli, A.1993. Rapid alteration of c-myc and c-jun expression in leukemic cells induced to differentiate by a butyric acid prodrug. FEBS Letters 328: 225-229.

Reddy, B. S. 1994. Chemoprevention of colon cancer by dietary fatty acids. Cancer Metastasis Reviews 13: 285-302.

Rodríguez-Alcalá, L. M. and Fontecha, J. 2010. Major lipid classes separation of buttermilk, and cows, goats and ewes milk by high performance liquid chromatography with an evaporative light scattering detector focused on the phospholipid fraction. Journal of Chromatography A 1217: 3063-3066.

Rombaut, R. and Dewettinck, K. 2006. Properties, analysis and purification of milk polar lipids. International Dairy Journal 16: 1362-1373.

Sanchez-Juanes, F., Alonso, J. M., Zancada, L. and Hueso, P. 2009. Distribution and fatty acid content of phospholipids from bovine milk and bovine milk membranes. International Dairy Journal 19: 273-278.

Tanmahasamut, P., Liu, J., Hendry, L. B. and Sidell, N. 2004 Conjugated Linoleic Acid Blocks Estrogen

Signaling in Human Breast Cancer Cells. Journal of Nutrition 134(3): 674-680.
Turpeinen, A. M., Mutanen, M., Aro, A., Salminen, I., Basu, S., Palmquist, D. L. and Griinari, J. M. 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. American Journal Clinical Nutrition 76: 504-510.
Valsta, L. M., Jauhiainen, M., Aro, A. and Katan, M. B. and Mutanen, M. 1992. Effects of a monounsaturated rapeseed oil and a polyunsaturated sunflower oil diet on lipoprotein levels in humans. Arteriosclerosis, Thrombosis and Vascular Biology 12: 50-57.
Vyas, D., Moallem, U., Teter, B. B., Fardin-Kia, A. R. K. and Erdman, R. A. 2013. Milk fat responses to butterfat infusion during conjugated linoleic acid-induced milk fat depression in lactating dairy cows. Milk fat response to short-and medium-chain fatty acids. Journal of Dairy Science 96(4): 2387-2399.
Walstra, P. and Jenness, R. 1984. Fatty acid composition. In Walstra, P. and Jenness, R. (Eds.). Dairy Chemistry and Physics, pp. 56-59. New York, USA: John Wiley and Sons.
Wat, E., Tandy, S., Kaper, E., Kamili, A., Chung, R.W.S., Brown, A., Rowney, M. and Cohn, J. S. 2009. Dietary phospholipid-rich dairy milk extract reduces hepatomegaly, hepatic steatosis and hyperlipidemia in mice fed a high-fat diet. Atherosclerosis 205: 144-150.
Watanabe, S., Takahashi, T., Tanaka, L., Haruta, Y., Shiota, M., Hosokawa, M. and Miyashita, K. 2011. The effect of milk polar lipids separated from butter serum on the lipid levels in the liver and the plasma of obese-model mouse (KK-Ay). Journal of Functional Foods 3: 313-320.
Witztum, J. L. and Steinberg, D. 1991. Role of oxidized low density lipoprotein in atherogenesis. Journal of Clinical Investigation 88: 1785-1792.
Wolff, R. L. 1994. Contribution of trans-18:1 acids from dairy fat to European diets. Journal of the American Oil Chemists' Society 71: 277-283.
Zock, P. L. 1995. Dietary fatty acids and risk factors for coronary heart disease: controlled studies in healthy volunteers. Wageningen, Netherland: University of Wageningen, PhD Thesis.
ZoÈllner, N. and Tato, A. F. 1992. Fatty acid composition of the diet: impact on serum lipids and atherosclerosis. Clinical Investigation 70: 968-1009.